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Study the flora in Marigold ensilage made from temple waste and evaluate the phytobiotic compounds developed.

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***Abstract*:** The primary causes of environmental degradation in a country could be attributed to the rapid growth of population, over-use of ecological resources, and establishment of different multinational companies and industries, which majorly affects the natural resources of the environment. Floral waste generated can become a threat to the environment and human beings. The rapid increase in the volume of waste is one of the significant phenomena leading to an environmental crisis. The main options available for processing/treatment or disposal of solid waste are composting, vermicomposting, anaerobic digestion, bio methanation, incineration, gasification, production of refuse-derived fuel, etc. There is a modern approach for converting floral wastes into value-added products namely compost, food products, biosurfactant production, incense sticks, etc. The present study deals with managing temple floral waste through efficient extraction of phytobiotic compounds developed during ensilage fermentation. Evaluation of the phytobiotic compounds using gas chromatography-mass spectrometry (GC-MS) was done. Around 30 compounds with a retention time of 2.02min to 35.072min were identified in GC-MS. More than 10% of these compounds are Neophytadiene 43.88%, 9,12,15-Octadecatrienoic acid-methyl ester 13.45% and hexadecanoic acid-methyl ester 13.24%.

Keywords: Marigold temple waste, Microorganisms, Ensilage, GC-MS analysis phytobiotic compounds.

I. INTRODUCTION

Environmental degradation is a significant threat confronting the world. Solid waste generally comes from residential and commercial areas such as houses, vegetable markets, hotels, marriages, palaces, hospitals, institutions, etc. Solid waste also includes the waste that is generally generated from religious practices in Temples, Mosques, Gurudwaras, etc. Devotees generally do not discard these waste flowers in dustbins, or any garbage because of religious beliefs. Devotees prefer to keep these flowers near trees, or dispose into water bodies

like rivers ocean etc. (*Patil et al 2016)* This practice leads to problems such as foul odor and land /water pollution. No tangible method has been used for the segregation of floral waste to date. (*Priyanka et al, 2011)*

In India, religion is a path of life. Worshiping is the way of living and people offer various offerings to their idols that mainly consist of flowers, leaves, fruits, coconuts, clothes, etc. Out of these flowers are present in large quantities. West Bengal is the fourth state position to promote flowers after Andhra Pradesh, Karnataka, and Chennai *(Yadav et al 2015).* Waste material weighing around 3.5- 4.0 tons is left behind every day in the city temples. Flower vendors often dispose flower wastes in the street, which could lead to outbreaks of serve endemic diseases as the garbage attracts pests. During the rainy season, the condition worsens with mosquitoes and flies breeding on the waste. The reason for offering flowers in large quantities in temples is, it produces a pleasant smell and creates an aesthetic environment that could help to reduce stress and anxiety. Next to plastics, waste flowers from temples create high impacts on the environment and human health. (*Yu-Yun Gao et al 2014*.) Hence, the treatment of floral waste is indispensable in the wake of urban development *(Rathika Govindasamy etal 2018).*

The main options available for processing solid waste are composting, vermicomposting, anaerobic digestion/ bio methanation, incineration, gasification or production of refuse-derived fuel (*Avintha et al 2015*). Ensilage is a technique for preserving forage, in which ensilage mass is acidified under anaerobic conditions. The lactic acid bacteria present in the environment produce lactic acid thereby making the environment acidic, and convert into soluble substrates like organic acids.

Figure-1: Collection and segregation of flower waste at a famous temple in Mumbai

II. MATERIALS AND METHODOLOGY

2.1Materials

Yellow and Orange marigold flowers were collected from some famous temples like Siddhivinayak Mandir, Mahalaxmi temple etc. Shitla Devi Mandir Sai Baba Mandir, Shriveer shakti Mandir, and Vitthal mandir were surveyed through questionnaires to understand the amount of floral waste generated and disposal policy if any, adopted by these temples. Media such as Nutrient agar, Sabouraud's Dextrose Agar, Carboxy methyl cellulose agar, tryptic soy agar, Czapek's agar, Malt agar, and Tomato juice agar were obtained from Himedia.

2.2 Isolation of Microbial flora present in marigold Ensilage fermentation

Yellow and Orange flower petals were mixed to make a homogenous sample and filled into screw-capped Scott Durham bottles. One set was mixed with 15 grams of soil and 350 grams of marigold petals, and another set was made with only 350 grams of marigold petals. Flask study was also carried with 25 grams of marigold petals (yellow, orange) added to 250 ml of Mineral broth. All the systems were incubated in the dark maintaining anaerobic conditions for 30days-240days and some upto a year. The microflora developed in the initial systems were studied by isolation on media including Nutrient agar, Sabouraud's Dextrose Agar, Tryptic soy agar, Czapek's agar, Malt agar, and Tomato juice agar. The plates were incubated under aerobic, anaerobic, and micro-aerophilic conditions. The isolates obtained from this initial fermentation were evaluated for their cellulose

degradation potential by spotting on CMC agar and using 0.1% Congo red & 1M NaCl by the method of *Jose et al 2003.*

2.3 Mixed Culture optimization for Ensilage fermentation.

In order to extract the phytobiotic compounds from the marigold petals, different sets of ensilages were prepared with the isolates obtained from the initial fermentation systems. One set of ensilage was made using bacterial cultures having an inoculum of 10^7 cells/ml. Another set of ensilage was made using only actinomycetes and fungal cultures having inoculum of 3.67×10^7 spores /ml. These ensilage systems were incubated under anaerobic conditions for one year. Subsequently phytobiotic compounds were evaluated after a solvent extraction method(*Lin et al 2015*).

2.4 Characterization of phytobiotic compounds by GCMS analysis (*Jaya et al 2017*)

The GC-MS was performed by HP 5ms columns Agilent's 30-meter. The column diameter was 0.25 mm and the thickness of the film 0,25µm. The carrier gas was Helium and ionizing used EL (70Ev).GC conditions: Column oven temperature 40°C, injector temperature 300°C by injection models are split with a ratio of 207.9. Flow control mode pressure 12 kPa. Column flow 0,56 mL/min, linear velocity 27,1 cm/sec. Mass spectrometric used under conditions Ion source temperature 250°C, interface temperature 300°C. Scan range 28-600m/z speed 1250, time scan starts at minute 0 to minute 50.

3. RESULTS & DISCUSSION

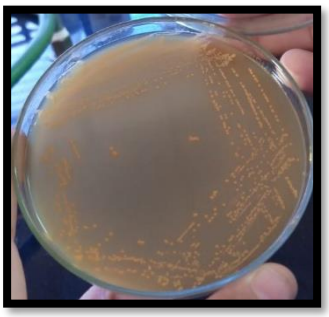
The marigold (*Tagetes erecta*) ensilage study commenced with a survey of waste marigold flowers generated in different temples across Mumbai (South Mumbai and Suburban Mumbai and Thane). This Survey was done to suggest a possible endeavor besides the existing strategy to tackle this vast flower waste generated especially in famous places of worship. Temples situated in South Mumbai, Suburban Mumbai & Thane were surveyed. Flower waste as low as 200kg to as high as 150,000kg is generated per day. During festive seasons the turnover is more.(*Patill et al 2015*) The temple authorities expressed concern about recycling this waste. Some were aware of its use in making dye, incense sticks, and composting. Improper management of this waste can cause odor, insects, and maggots infestation.

3.1Microbial flora present in marigold Ensilage fermentation

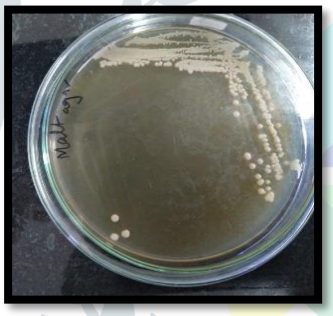
Many bacterial isolates and actinomycetes isolates were obtained, identification of these isolates was done only to gram nature and morphology The liquor from the fermented marigold ensilage (Figure-2) was studied for the developed flora by isolating on different media (Figure 3) and incubating under different conditions of air. Gram nature and morphology detected Gram positive cocci , coccoid, rods, yeast cells. A conspicuous noting were magenta red colored colonies. Out of the 20 isolates obtained from the initially fermented ensilage, 17 isolates showed cellulase activity .Figure 3 shows different growth pattern of isolates obtained from the fermented marigold.

Marigold petals With soil | Marigold petals without soil | Marigold petals with soil | Marigold petals without soil

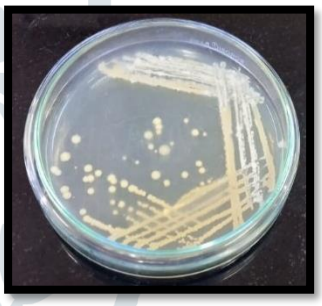
Figure-2: Fermented marigold flowers after one year



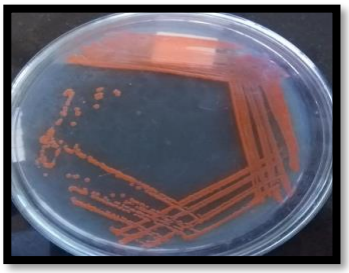
Gram positive cocci



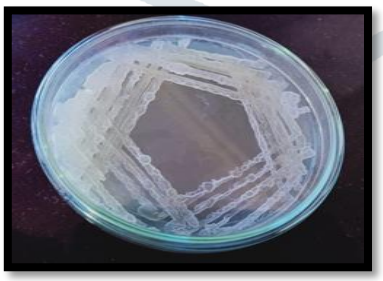
Gram positive yeast



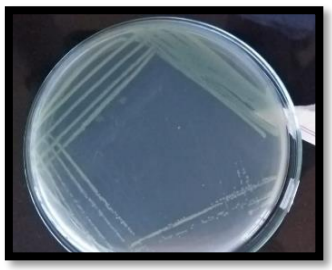
Gram positive rods



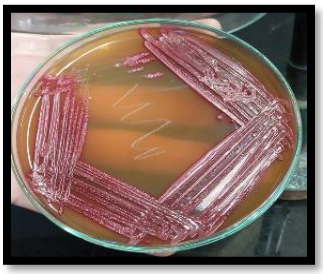
Gram positive rods



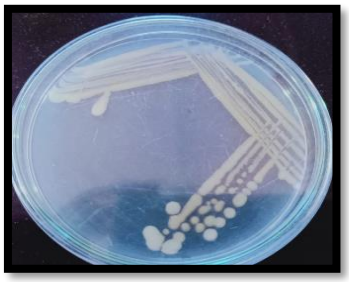
Gram positive filamentous



Gram negative rods



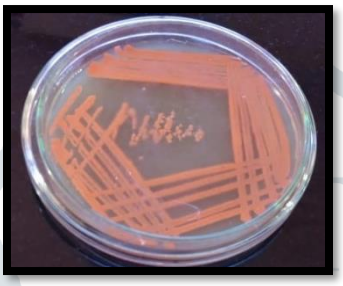
Gram negative cocci



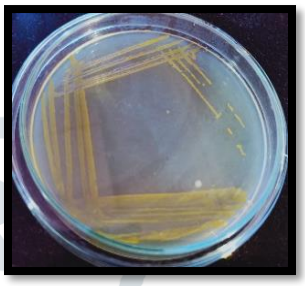
Gram positive coccoid

Septate mycelium

Gram positive cocci



Gram negative rods



Gram positive cocci

Figure 3 : Isolates obtained during marigold fermentation

3.2Mixed Culture optimization for Ensilage Preparation

The effective ensilage fermentation was set up again using the microflora obtained in the initial systems. Selective isolates were subsequently developed in pure broth culture and used as a starter for the scale-up ensilage fermentation as done by *Bolonas etal 2005*. The fungal, bacterial and actinomycetal cultures were grown in pure cultures and different consortiums were prepared. These were added to the bottled marigold petals. After one year it was found that enzymatic reactions that are done by bacteria and fungi helped in the degradation of cell–wall components such as cellulose, hemicellulose etc. *Sowbhagya et al 2014* indicated the increased extraction of xanthophylls and other phycobiotic content due to enzymatic pretreatment during extraction of marigold flower. The cell walls presumably exhibited enough amount of permeability that helps in mass transfer of hydro-soluble cell components between solid petals and liquid extracts. Due to increase in cell wall permeability extraction of phytobiotic compounds could be effectively done. Due to the prolonged ensilage the xanthophyll also got extracted into the fermented liquor. The bacterial & fungal consortiums gave more effective degradation of marigold petals and release of phytobiotic compounds.

Bacterial ensilage 1& 2 with an inoculum of 1×10^{7}cells/ml

Bacterial fermented Ensilage (After one year)

Fungal Ensilage 3 & 4 with an inoculum of 3.67×10^{7}cells/ml

Fungal Fermented Ensilage (After one year)

(Figure-4: Mixed culture optimization study for ensilage fermentation)

3.3 Phytobiotic compound extraction from marigold flowers:-

All the dried sample of the fermented marigold were extracted with acetone and hexane to achieve high amount of phytobiotic compounds [described by *Lin et al 2015*]. The result showed in figure 5 indicates bacterial ensilage that was extracted in hexane given less amount of phtyobiotic compounds as compared to fungal ensilage extracted in acetone

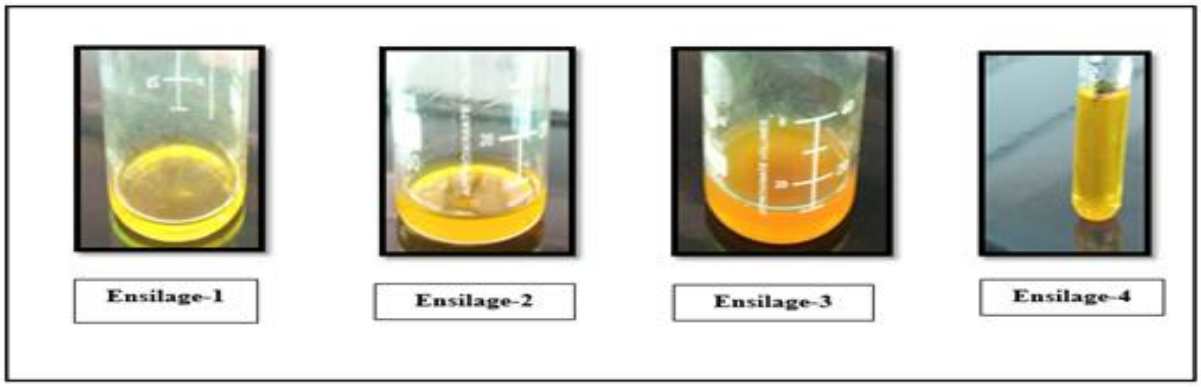


Figure-5 Extraction of phytobiotic compounds from bactieral & fungal fermented ensilage held under anaerobic condition for 1 year in the dark

3.4 Characterization of phytobiotic compounds by GCMS analysis

In order to evaluate the phytobiotic compounds present in the marigold petals after ensilage fermentation, the acetone and hexane extract were subjected to GC-MS analysis. . Literature review finds about 95% of lutein, Xanthophyll, Carotenoids, Zeaxanthin, present in the flowers is in the form of esters out of which lutein palmitate is the major pigment (*Gau et al. 2014*). For hexane, extract compounds are listed in table -1 and for acetone extract, compounds are listed in table -2

There are total 30 compounds analyzed by GC-MS. The first compound at a retention time of 2.224 was Alanine ethylamide . The last compound with a retention time of 35.72 Propanamide, 3,3,3-trifluoro-2-(trifluoromethyl) ,Apart from this there were different phytochemicals were analyzed which can be used as an antibacterial , antifungal agent etc.

Many esters have been found in the GCMS analyte. Oxidation products of xanthophyll are mono and di-epoxides, carbonyls, alcohols. Many compounds detected in the GCMS show promising health benefits and antibacterial effects (*Chen et al 1992*), the ensilage extract can perhaps also be used as agricultural sprays to perhaps kill phyto-pathogens, a study for the future. Liquor obtained or fermented marigold flowers need to be investigated in the aquarium for improving the pigment in aquarium fishes, a study for future.

Table -1: Phytobiotic compounds extracted from bacterial ensilage using hexane solvent

Peak	Retention time (min)	Area %	Height	Name
1)	2.016	11.76	12710075	9-Octadecenoic acid (Z)-, 2-hydroxy-3-[(1-oxohexadecyl) oxy]propyl ester
2)	2.064	12.82	2163890	Octadecanoic acid, octadecyl ester
3)	10.019	7.23	1000576	Nonanoic acid

4)	14.749	7.54	2280626	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)
5)	16.645	5.58	1784727	Santalol, cis,.alpha.acid
6)	20.589	6.38	5394311	n-Hexadecanoic acid
7)	23.307	7.80	6590404	9-Hexadecenoic acid

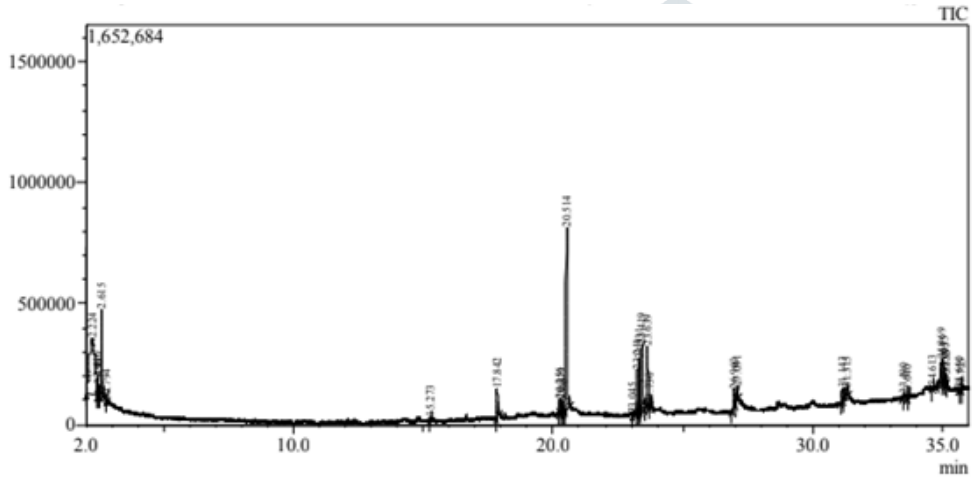


Figure 6 : GCMS analysis of Phytobiotic compounds identified in Hexane extract of bacterial ensilage.

Table -2: Phytobiotic compounds extracted from fungal+ Actinomycetes) ensilage using acetone solvent

Peak	Retention time	Area %	Height	Name
1)	2.224	25.62	233801	l-Alanine ethylamide, (S)- 2-Amino-N-ethylpropanamide
2)	2.615	7.95%	10641606	CompName:(2-Aziridinylethyl)amine, Aziridine, 1-(2-aminoethyl)- N-(.beta.-Aminoethyl)ethylenimine
3)	20.514	15.44	758064	n-Hexadecanoic acid

4)	23.310	7.00	221169	cis-Vaccenic acid
5)	23.419	7.80	279095	Bis(2-ethylhexyl) maleate
6)	23.639	6.72	258485	Octadecanoic acid
7)	26.960	2.34	77286	cis-11-Eicosenoic acid
8)	27.091	1.87	63595	9-Hexadecenoic acid
9)	34.969	4.45	93848	Octanoic acid, 4-pentadecyl ester
10)	35.055	1.59	66587	Acetic acid, (2-amino-6H-[1,3,4]thiadiazin-5-yl)

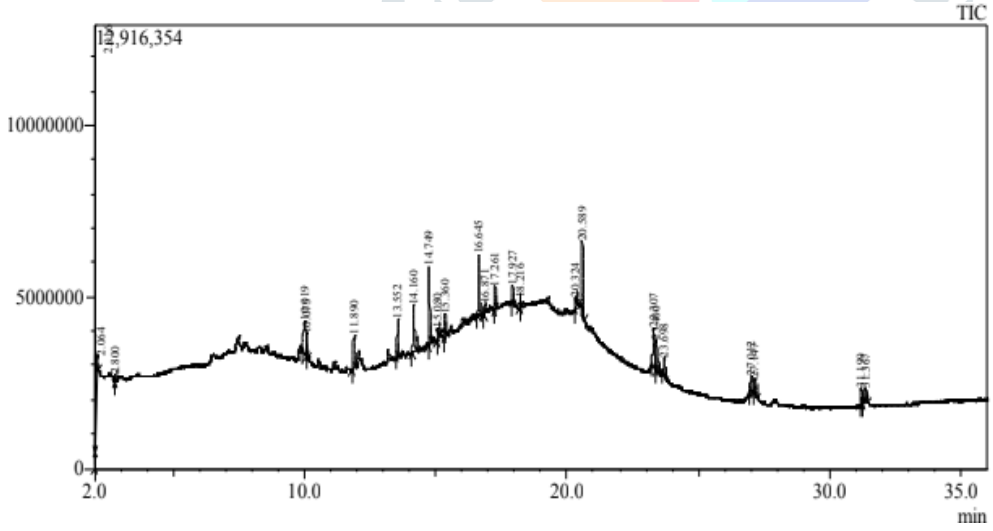


Figure 7 : GCMS analysis of Ensilage 4 phytobiotic extract
GCMS analysis of Phytobiotic compounds identified in acetone extract of fungal ensilage.

4. CONCLUSION

India is rapidly shifting from an agricultural-based nation to an industrial and services-oriented country. Waste is a natural by-product of the phenomena of life and the growth of societies. Next to plastic, waste flowers from temples create a high impact on the environment & Human health. The present study describes the management of floral waste into a valuable product. Temple survey was carried out to understand the reality of the amount of floral waste generated in popular temples in Maharashtra.30 types of compounds with a retention time of 2.02 to 35.072. Compounds containing more than 10% are Neophytadiene 43.88%, 9,12,15-Octadecatrienoic acid-

methyl ester 13.45% and hexadecanoic acid-methyl ester 13.24%. The degraded material has a high value of N, P, and K content so it can be used as a biofertilizer & feed for animals. In order to reduce the volume of waste flower generation, awareness should be created among devotees & temple authorities about the improper disposal of waste flowers. Hence more research needs to be carried out to understand the importance of temple waste marigold flowers.

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